Supplementary information, Figure S4

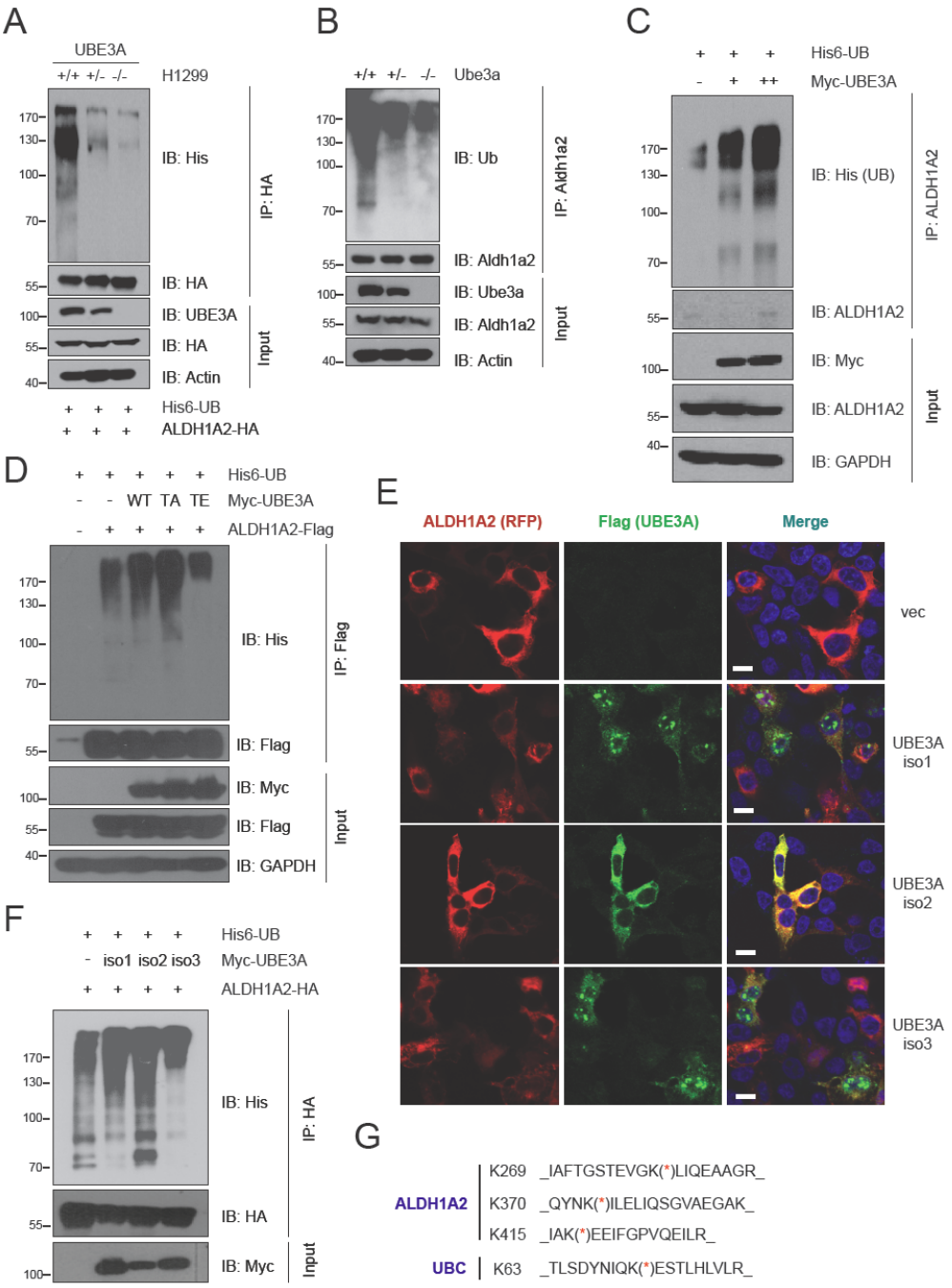

Figure S4 UBE3A ubiquitylates endogenous or exogenous ALDH1A2 protein in a dosage-dependent manner. (**A**) The ubiquitylation level of ALDH1A2 was decreased

upon depletion of UBE3A, in H1299-derived cells with indicated *UBE3A* genotypes (+/+, +/-, and -/-). The cells of different genotypes were co-transfected with constructs encoding His-Ub and ALDH1A2-HA constructs. ALDH1A2-HA proteins were enriched and analyzed by IB using anti-His antibody to detect the conjugated poly-Ub chains on ALDH1A2. (**B**, **C**) Ubiquitylation of endogenous Aldh1a2 protein was correlated with the dosages of Ube3a proteins in MEF cells of indicated genotypes **(B)** or HEK-293FT cells **(C)**. Endogenous Aldh1a2 protein was immunoprecipitated from MEF cells with anti-Aldh1a2 antibody, followed by IB with anti-Ub antibody. HEK-293FT cells expressing His6-Ub were transfected with Myc-UBE3A constructs at increasing dosages, from which endogenous ALDH1A2 protein was immunoprecipited and subjected to IB with anti-His antibodies to detect the conjugated poly-Ub chains on ALDH1A2. (**D**) The phosphorylation status of UBE3A affected its E3 ligase activity towards ALDH1A2 substrate. HEK-293FT cells were co-transfected with constructs encoding His-Ub, ALDH1A2-Flag and wild-type (WT) UBE3A or its mutants of indicated mutations (TA, T508A; TE, T508E). Ubiquitylation of ALDH1A2-Flag was detected by enriching ALDH1A2 with anti-Flag antibodies, followed by IB with anti-His to detect the conjugated poly-Ub chains on ALDH1A2. (**E**) Differential localization of UBE3A three isoforms using immunofluorescence analysis. SH-SY5Y cells were co-transfected with Flag-tagged human UBE3A isoforms and ALDH1A2-RFP fusion plasmids. Cell nucleus was stained in blue with DAPI dye. Scale bar, 10 μm. (**F**) Differential ubiquitylation activities upon the substrate ALDH1A2 for three isoforms of UBE3A. HEK-293FT cells

were co-transfected with human UBE3A isoforms, ALDH1A2-HA and His-UB. Ubiquitylation level of ALDH1A2 was examined using immunoprecipitation and immunoblotting assay. (**G**) Sequences of the peptide containing the ubiquitylation residues in ALDH1A2 and Ub lysine-linkages were identified by mass spectrometry analysis, while the asterisk in red color highlighted the ubiquitylation modified lysine residue. These modified residues were the convergent sites identified in enriched proteins from both transfected HEK-293FT cells and reconstituted bacterial strains. All experiments were conducted at least three times.